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Plant, microbial community and soil property responses to an experimental precipitation gradient in a desert grassland

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ABSTRACT

Major changes in precipitation regimes can be predicted, but the effects of such changes on terrestrial ecosystem functioning are largely unknown. To investigate the effects of changes in precipitation regimes on the plant community, soil properties, soil respiration and bacteria community composition in an arid grassland, we conducted a three-year precipitation manipulation experiment (i.e., ambient precipitation as a control, ± 20% and ± 40% of ambient precipitation) in a desert grassland in the western Loess Plateau, China. The species richness, density, mean height, aboveground biomass and litter biomass were reduced in the −40% precipitation treatment. Soil water content at a depth of 0–20 cm peaked in the +40% precipitation treatment. The influences of precipitation treatment on dissolved organic carbon (DOC), microbial biomass carbon (MBC) and soil nutrients were small. Soil respiration varied along the experimental precipitation gradient and peaked in the +40% precipitation treatment. NO_3^--N was highest in the −40% precipitation treatment. *Bacteroidetes* had a higher relative abundance in the increased precipitation and control groups, while the abundance of *Actinobacteria* was higher in the decreased precipitation treatment in the wet year. The differences among precipitation treatments were not detected in the dry year. Thus, increased soil respiration along the precipitation gradient resulted from the positive responses of root growth, reflected in the plant community properties, and microbial respiration, reflected in the bacterial community composition. Different responses of ecosystem components to precipitation manipulation emphasize the necessity of studying the relationships between these components under climate change.

1. Introduction

Anthropogenic emissions of greenhouse gases are expected to cause significant changes in global climate in this century (IPCC, 2013). It has been suggested that climatic warming alters the amount and distribution of precipitation by increasing the water-holding capacity of the atmosphere, enhancing the evaporation rate and disrupting air circulation patterns (Trenberth, 2011). This had led to intensified intra- and inter-annual variations in precipitation in recent years (Min et al., 2011; Donat et al., 2013). Shifts in precipitation regimes, especially in arid and semi-arid environments where water determines primary production, may have an even greater impact on ecosystem dynamics than the singular or combined effects of rising CO_2 and temperature (Loarie et al., 2010). A better understanding of the effects of increased or decreased precipitation on the structure and function of ecosystems is critical for predicting how ecological services will change under future climate change scenarios.

A change in the precipitation amount would affect both the temporal and spatial distribution of water availability and potentially alter the structure and function of biotic communities (Tomiolo et al., 2015). Changes in precipitation patterns could also change the diversity, primary productivity and functional composition of plant communities, and ultimately alter the quantity and quality of carbon (C) inputs into soils (Hooker et al., 2008). However, small or delayed responses of plant community structure to long-term precipitation manipulation have been detected in water-limited temperate grasslands (Grime et al., 2000; Collins et al., 2012; Tielbörger et al., 2014). These were attributed to the tremendous temporal and spatial heterogeneity under which the plants have evolved.

The labile organic carbon pool is the most active fraction of soil organic matter and acts as a direct reservoir of readily available nutrients for plants and microbes (Schimel et al., 2007). It exerts considerable control on soil C flux and ecosystem functioning (Allison and Treseder, 2008). These have been shown to be sensitive to changes in management practices (Rui et al., 2011). However, there is uncertainty over the effect of altered precipitation on dissolved organic carbon

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(DOC) and microbial biomass carbon (MBC). Some studies have found that increased precipitation could increase DOC and MBC (Zhang et al., 2013; Zhou et al., 2013; Huang et al., 2015; Zhao et al., 2016), and others have observed decreased MBC after increased precipitation (Sherman et al., 2012; Zhang and Zak, 1998). Insignificant differences in MBC (Cregger et al., 2014) and DOC (Sherman et al., 2012) were also found between altered precipitation levels. Therefore, uncertainties still exist in the feedbacks of the soil C pool in response to varied water availability under global change scenarios.

Microorganisms play key roles in soil biogeochemical processes, including organic matter decomposition and nutrient mineralization (Cregger et al., 2014). A higher precipitation is reported to increase the availability of respiratory substrates (Zhou et al., 2013), which could also change microbial C use and stimulate microbial growth and physiological activities (Zhou et al., 2013; Zhao et al., 2016). However, some studies have seen moisture-related changes in soil microbial community composition (Fierer et al., 2007; Barnard et al., 2013; Maestre et al., 2015), and others have observed few or no differences in the microbial community structures of wet and dry soils (Landesman and Dighton, 2010; Zhang et al., 2013; Curiel Yuste et al., 2014). More evidence concerning microbial community composition is necessary for understanding the general response of soil microorganisms to a greater variability in precipitation.

To investigate the effects of precipitation on plant communities, the labile organic C pool, soil respiration and microbial composition, a field experiment with five levels of precipitation manipulation (i.e., ambient precipitation as a control, ± 20% and ± 40% of ambient precipitation) that cover the natural range in precipitation variation was conducted in a temperate desert grassland in the western Loess Plateau starting in 2013 for three years. Given the known differences in the responses of ecosystem components to prolonged precipitation manipulation, we hypothesized the following: 1) Species richness and community biomass would increase with increasing precipitation and decrease with decreasing precipitation, 2) DOC, MBC and mineralized nitrogen would increase with increasing precipitation, and 3) Microbial community compositions would differ among precipitation treatments.

2. Materials and methods

2.1. Site description and experimental design

The experimental sites are at the Gaolan Experiment Station for Ecology and Agriculture Research, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences. The station (36°13″ N, 103°47″ E) is located in Gaolan county, Lanzhou city in Gansu province, northwest of the Loess Plateau. The altitude is approximately 1780 m. The climate is a drought-prone, semi-arid, continental climate. The average annual precipitation is 263 mm with 70% falling between May and September. The mean annual temperature is 8.4 °C, with a maximum mean monthly temperature of 20.7 °C (July) and a minimum mean monthly temperature of −9.1 °C (January). Mean annual pan evaporation is 1786 mm. The average soil organic carbon and total nitrogen are 0.75% and 0.1%, and pH is 8.52. The soil in this region is developed from wind-accumulated loess parent material, with a uniform silt loam texture and is classified as a *Haplic Calcisol* in the FAO/UNESCO classification system. The site is dominated by typical desert steppe vegetation, including the semi-shrub *Ajania fruticulosa* (Ledeb.) Poljak, *Reaumuria soongarica* (Pall.) Maxim and perennial grass *Stipa breviflora* Griseb. Common perennial herbaceous species include *Peganum harmala* L., *Zygophyllum mucronatum* Maxim, *Artemisia capillaris* Thunb and *Cleistogenes squarrosa* (Trin.) Keng. Annual herbaceous plants are less abundant and the most common species is *Salsola ruthenica* Iljin (Supplementary Table S1).

The experiment used a randomized complete block design with five treatments: −40% and −20% of the ambient precipitation, ambient precipitation as a control, and +20% and +40% of the ambient precipitation. The inter-annual precipitation variation was from −41.1% to +39.2% over the past 50 years in the study area. Precipitation frequency and timing were not changed within our treatments. Each treatment was replicated three times, and each replicate plot was 2.5 × 2.5 m^2. The precipitation treatments were applied from May to September each year from 2013 to 2015.

The rainout shelter is consisted of a fixed-location shelter with a roof made of transparent acrylic bands that block different amounts of rainfall and minimally affect other environmental variables (Yahdjian and Sala, 2002). The mean height of the shelter was 0.50 m. Light transmittance through the acrylic is very high compared to plastic or PVC, and the amount of photosynthetically active radiation intercepted is less than other shelter designs. Greenhouse effects are possibly eliminated due to the unconstrained air movement. The appropriate volume of rain intercepted in the decreased precipitation plots was added to the increased precipitation plots manually within 8 h of each precipitation event.

2.2. Plant community investigation

Two frames (0.5 × 0.5 m), each with 50 equally distributed grids, were placed above the canopy at the center of each plot and were used to measure plant species richness, density of each species, height and coverage once a month from late May to late September in 2013, 2014 and 2015. Species richness was recorded as the number of plant species in the quadrat. Density was calculated as the sum of the individual numbers for all species. The mean height was the average value of all species' heights. Meanwhile, the senescent litters of the two subplots were collected and oven-dried to obtain a biomass measurement.

Aboveground biomass was assessed using a harvesting method at the time of peak biomass (early September). All plants were clipped to the soil surface by species in another two quadrats (0.5 × 0.5 m) in each plot. After oven drying for 48 h at 65 °C, the dry mass was weighed to determine the biomass (g m^{-2}) of each species. Community aboveground biomass (g m^{-2}) was estimated from the sum of all species' aboveground biomass. The aboveground biomass was sampled in the same two quadrats in the first 2 years and in another two quadrats in the third year.

2.3. Soil respiration measurement

To measure soil respiration, two PVC collars (11 cm in internal diameter and 5 cm in height) were inserted 3 cm into the soil at two opposite corners in each plot. All living plants inside the soil collars were removed by hand at least 1 day prior to the measurements. Soil respiration was measured using an LI-6400-09 soil chamber (Li-Cor, Inc., Lincoln, NE, USA). The observation length was 2–3 min on each collar. The values of the two collars in each plot were averaged as one replicate. Soil respiration was measured every 2 h between 8:00 and 18:00 from June-September in 2013, 2014 and 2015.

2.4. Soil sampling and edaphic properties

Soil samples were collected from all 15 plots on August 15th of 2013, 2014 and 2015 and June 15th of 2014. In each plot, three soil cores at each depth (0–5, 5–10 and 10–20 cm depth; 5 cm diameter) were randomly taken using an auger and were mixed to obtain one composite sample per plot. After visible roots and stones were removed by hand, the soil was then passed through a 2 mm sieve. Subsamples passing through a 1 mm sieve were placed in an icebox and transported to the laboratory for microbial analysis. Other subsamples were air-dried for chemical analysis.

Dissolved inorganic nitrogen (NH_4^+-N and NO_3^--N) was extracted from 10 g of fresh soil with 50 ml of 2 M KCl and was measured with a Flow Injection Analyzer (Skalar, Breda, the Netherlands) (Zhao et al., 2016). Soil microbial biomass carbon and nitrogen (MBC and MBN)

were measured using the fumigation-extraction method (Brookes et al., 1985). The organic carbon in un-fumigated soil extracts was used as dissolved organic carbon (DOC) (Zhao et al., 2016).

Soil organic carbon (SOC) was measured using the $K_2Cr_2O_7$–H_2SO_4 oxidation method (Institute of Soil Sciences, Chinese Academy of Sciences (ISSCAS), 1978). Total nitrogen (TN) was measured with the micro-Kjeldahl procedure and available Phosphorus (AP) was determined by the Bray method (ISSCAS, 1978). In addition, soil pH was determined by a glass electrode and the electrical conductivity (EC) was determined by an EC meter with a 1:2.5 soil:water ratio (ISSCAS, 1978).

Soil water content (SWC) at depths of 0–20, 20–40, 40–60, 60–80, 80–100, 100–120, 120–140, 140–160 and 160–180 cm in 2013, 0–5, 5–10, 10–20, 20–40 and 40–60 cm in 2014 and 0–5, 5–10, 10–20, 20–40, 40–60, 60–80, 80–100, 100–120, 120–140, 140–160 and 160–180 cm in 2015 were measured using the oven-drying method. Soil temperature (ST) at a 5 cm depth was determined using a thermocouple probe connected to the LI-6400-09 soil chamber (Li-Cor, Inc., Lincoln, NE, USA).

2.5. Soil microbial community analysis

The soil microbial community composition was analyzed in August 2014 and 2015. The soil genomic DNA was extracted directly using a soil DNA extraction kit (Omega Bio-Tek, GA, USA) according to the manufacturer's instructions. The concentration and purity of DNA extracts were determined using a NanoDrop ND-2000C spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Qualified total genomic DNA was amplified using 515F/806R primers, which amplify the V4 region of the 16S rDNA gene, to determine the diversity and composition of the bacterial communities in each sample. DNA was amplified according to a previously described protocol (Caporaso et al., 2011). High-throughput sequencing of amplicons was conducted using the Illumina MiSeq platform at Novogene Bioinformatics Technology Co. Ltd. (Beijing, China). The mean sequencing depth of individual samples were 47,952 clean reads (from 14,672 to 67,849) in 2014 and 59,822 clean reads (from 55,331 to 63,943) in 2015, respectively. Complete datasets from this study have been deposited in the NCBI Short Read Archive database under accession numbers SRR4232079.

Pairs of reads from the original DNA fragments were merged using FLASH. Sequencing reads were assigned to each sample according to the unique individual barcodes. Sequences were analyzed with the QIIME software package (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010) and UPARSE pipeline (Edgar, 2013). The operational taxonomic units (OTUs) were detected at 97% similarity. A representative sequence was selected for each OTU and used to assign taxonomic composition with the RDP classifier.

2.6. Statistical analysis

The effects of year, precipitation (i.e., precipitation treatments) and their potential interactions on SWC, ST, plant species richness, density, mean height, aboveground biomass, litter biomass, soil respiration, DOC, MBC, MBN, ratio of MBC to MBN (MBC/MBN), NO_3^--N, NH_4^+-N, and soil properties such as SOC, TN, AP, pH, EC and ratio of SOC to TN (C/N) were analyzed using two-way ANOVAs. Moreover, multiple comparisons were used to examine the effects of the five precipitation treatments on each of these variables in each year. These ANOVAs were performed using SPSS 15.0 (SPSS for Windows, Version 15.0, Chicago, IL). In addition, Redundancy Analyses (RDA) was performed using Canoco 4.5 to evaluate the relationship between microbial community composition and plant-soil environmental factors.

Table 1
Results (F-values) of two-way ANOVAs for the effects of year (Y), precipitation treatment (P) and their interactions (Y × P) on soil water content (SWC), soil temperature (ST).

Source of variation	SWC			ST		
	0–20 cm	20–40 cm	40–60 cm	8:00	11:00	15:00
Y	87.38***	14.45***	4.27*	22.58***	14.23***	37.80***
P	5.48**	1.59	1.74	0.39	0.16	1.51
Y × P	0.38	0.10	0.27	0.40	0.81	0.52

3. Results

3.1. Effects of precipitation change on soil moisture and temperature

The ambient precipitation was different between years (Fig. S1a) with 235, 252.7 and 152.5 mm falling between May and September in 2013, 2014 and 2015, respectively, and occupying 92%, 71% and 80% of the annual precipitation, respectively. In addition, the winter air temperature was lowest in 2013, intermediate in 2014 and highest in 2015 with a difference of 2 °C, and was also higher in other seasons in 2013 (Fig. S1b).

Precipitation treatment influenced soil water content (SWC) at a depth of 0–20 cm (Table 1, Fig. 1). SWC peaked in the +40% precipitation treatment ($p < 0.01$). SWC at deeper depths did not vary along the precipitation gradient. However, SWC varied with year ($p < 0.05$; Table 1). It was higher in 2014 (7.2%) than in 2013 (4.9%) and 2015 (3.3%) in the 0–20 cm soil depth (Fig. 1). However, no significant effects of precipitation on soil temperature at a 5 cm depth were found (data not shown).

3.2. Plant growth and biomass response to precipitation alteration

The species richness, density, mean height and aboveground biomass increased in the increased precipitation treatments across all three years ($p < 0.05$, 0.01 and 0.001; Fig. 2). Moreover, the difference in these properties between increased and decreased precipitation treatments was greatest in 2015 relative to 2013 and 2014, reaching nearly 3 times the density and 1.5 times the mean height in 2015.

Across the 3 years, litter biomass was higher in the +40% precipitation treatment than in the −40% precipitation treatments ($p < 0.05$; Fig. S2). Meanwhile, the inter-annual change and the interaction between precipitation treatment and years was also significant (Fig. S2).

3.3. Effects of precipitation alteration on soil respiration and the labile carbon and nitrogen pools

Soil respiration varied along the experimental precipitation gradient and peaked in the +40% precipitation treatment ($p < 0.001$; Fig. 3). It also varied with year and was higher in the wet year (2014) than in the dry year (2015; $p < 0.001$).

DOC, MBC and MBN did not vary along the precipitation gradient, while MBC/MBN at the 0–5 cm depth peaked in the −40% precipitation treatment ($p < 0.05$; Fig. 4). Moreover, inter-annual variations in DOC, MBC and MBC/MBN were detected, and they were higher in the dry year (2015) than in the wet year (2013 and 2014) ($p < 0.05$–0.001).

Precipitation treatment influenced the NO_3^--N content, which peaked in the −40% precipitation treatment during all 3 years ($p < 0.01$–0.001; Fig. 5). Meanwhile, NO_3^--N increased with increasing soil depth. However, NH_4^+-N content did not vary with precipitation treatment but did vary by year ($p < 0.05$; Fig. 5).

Precipitation treatment had significant effects on some soil properties (Fig. S3). EC at the 0–10 cm soil depth accumulated in the −40% precipitation treatment. Meanwhile, the highest TN and AP were in the

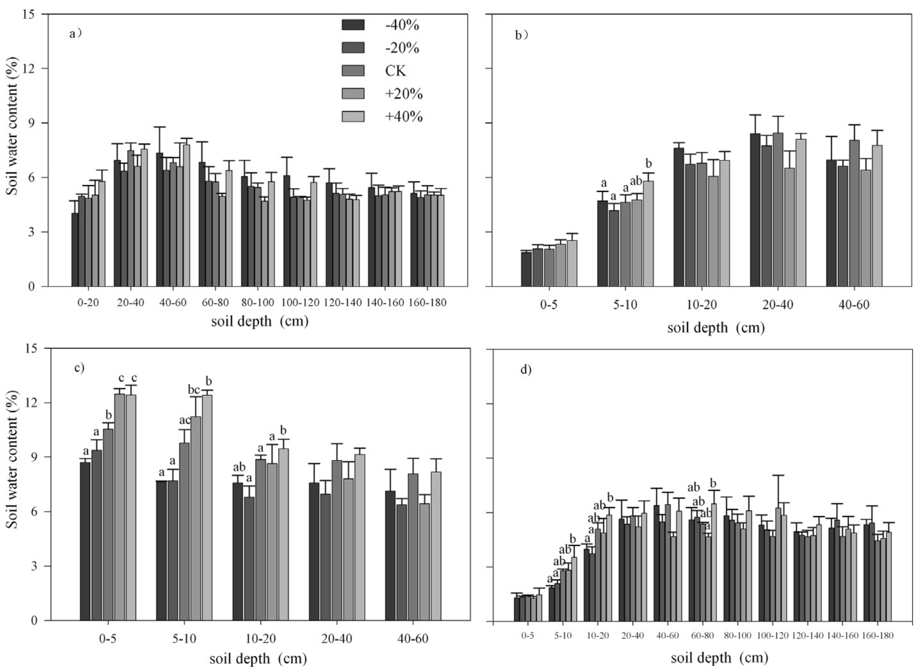

Fig. 1. Soil water content after 3 years of precipitation alteration (mean ± SE, n = 3, main panels). Bars with different letters within each soil depth represent significant differences based on post hoc testing at $p < 0.05$. −40%: 40% decrease in precipitation, −20%: 20% decrease in precipitation, CK: control, +40%: 40% increase in precipitation, +20%: 20% increase in precipitation. a: 2013, b: June in 2014, c: August in 2014, d: 2015.

decreased precipitation and control groups and the highest pH was in the increased precipitation treatment. Additionally, there were inter-annual variations in SOC, AP, pH and C/N, which were higher in the wettest year ($p < 0.05$–0.001; Table S3).

3.4. Microbial community composition response to precipitation alteration

In the wet year (2014), 76.5% of the total relative abundance was made up of *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* and *Acidobacteria* phyla (Table 2). The relative microbial abundance differed among precipitation treatments. For the *Bacteroidetes* phylum, the relative abundance was higher in the +40% precipitation and control groups than in the decreased precipitation group. Additionally, the relative abundances of *Actinobacteria* and *Gemmatimonadetes* were higher in the decreased precipitation groups than the increased precipitation groups.

In the dry year (2015), the bacterial communities were dominated by *Proteobacteria*, *Actinobacteria* and *Acidobacteria*, with a total relative abundance of 75.3%. *Bacteroidetes* was not part of the dominant phyla, with its abundance declining from 20.6% in 2014 to 1.5% in 2015. Significant differences in relative abundances of phyla among precipitation treatments were not detected.

Furthermore, compared with the wet year, the relative abundances of most phyla increased in the dry year, except for *Bacteroidetes* and *Verrucomicrobia* which declined and *Acidobacteria* which showed little change.

The relationship between the soil microbial community composition and environmental factors varied in two years (Fig. 6). Eigenvalues of RDA indicated that axes 1 explained 84.7% of the overall variance in 2014, while they explained 92.4% in 2015 within the soil microbial community composition among the treatments. In 2014, along the RDA1 (from left to right), the manipulated precipitation amount increased and the relative microbial abundance increased (e.g. *Bacteroidetes*). While the relative abundance of *Actinobacteria,*

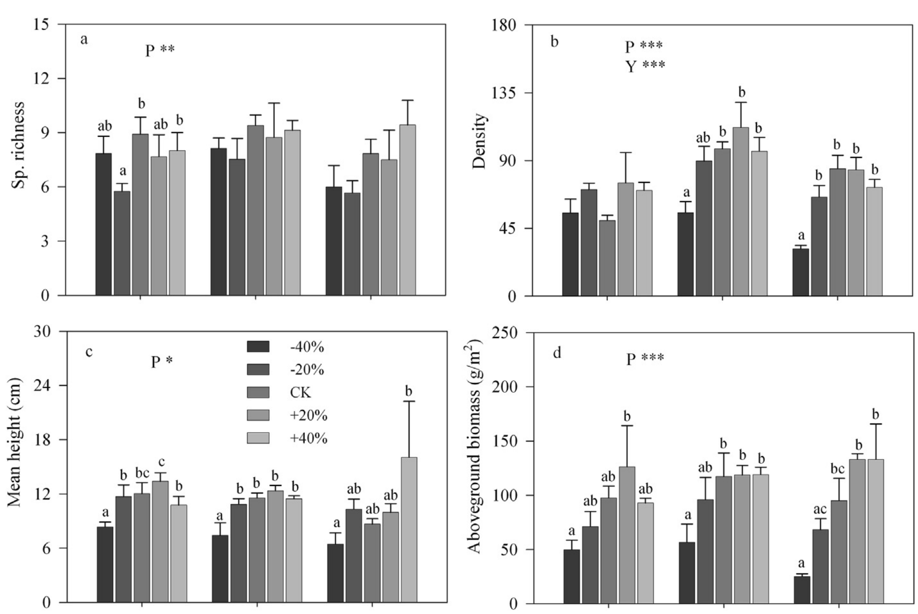

Fig. 2. Mean plant growth and biomass by year (mean ± SE, n = 5, main months) after precipitation alteration. Significant results of the two-way ANOVA on the effects of year (Y) and precipitation treatment (P) are shown. Significance: $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$. Bars with different letters within years represent significant differences based on post hoc testing at $p < 0.05$. −40%: 40% decrease in precipitation, −20%: 20% decrease in precipitation, CK: control, +40%: 40% increase in precipitation, +20%: 20% increase in precipitation. Inds: individuals.

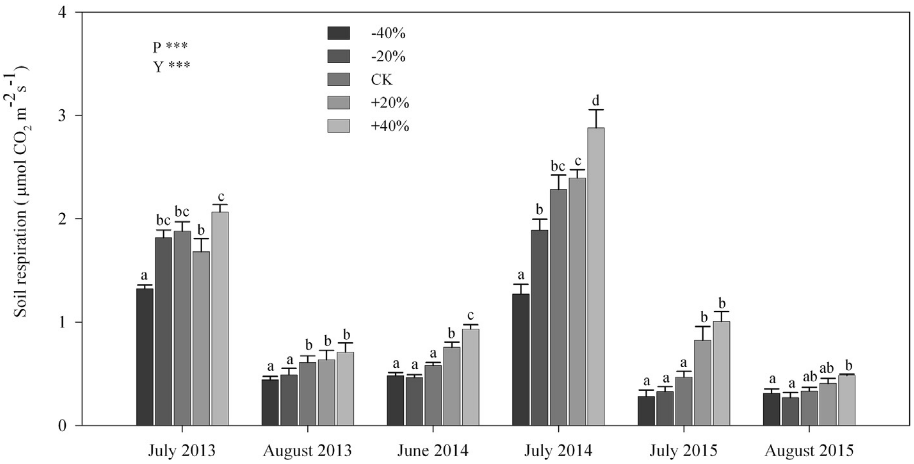

Fig. 3. Soil respiration (mean ± S.E., n = 4, main time periods) after changes in precipitation. Significant results of the two-way ANOVA on the effects of year (Y) and precipitation treatment (P) are shown. Significance: $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$. Bars with different letters within years represent significant differences based on post hoc testing at $p < 0.05$. −40%: 40% decrease in precipitation, −20%: 20% decrease in precipitation, CK: control, +40%: 40% increase in precipitation, +20%: 20% increase in precipitation.

Gemmatimonadetes and *Chloroflexi* were higher in the decreased precipitation treatments (Fig. 6a). Thus, RDA1 was highly correlated to TN, richness, SOC and SWC in 2014, with correlation coefficients of 0.92, 0.91, 0.86 and 0.77, respectively (Fig. 6a). In 2015, the pattern of relative microbial abundance among the precipitation treatments was not obvious (Fig. 6b). MBC, MBN and mean height were negatively with RDA1 with correlation coefficients of -0.98, -0.97 and -0.6, respectively in 2015 (Fig. 6b).

4. Discussion

In general, precipitation falling during summer months is subject to evaporation and transpiration, thus limiting percolation into the deeper soil profile (Sala et al., 1997). In fact, increased precipitation treatments increased SWC at a 0–20 cm soil depth in this study. Correspondingly, due to the numerous shallow rooted grasses and forbs, the species richness, density, mean height and aboveground biomass reduced in the −40% precipitation treatment, which proved the hypotheses 1. Soil respiration varied along the experimental precipitation gradient and peaked in the +40% precipitation treatment. However, DOC, MBC and soil nutrient were not significantly influenced by the precipitation alteration of three years, which disproved the hypotheses 2. Furthermore, the difference in microbial community compositions among precipitation treatments was detected in the wet year, which partly proved the hypotheses 3.

4.1. Response of plant community properties to precipitation alteration

In this study, precipitation treatment significantly influenced the species richness, density, mean height and aboveground biomass across the 3 years. The results reported here were not consistent with Tielbörger et al. (2014), who found few differences in the biomass, species richness and density between irrigation and drought treatments across nine years of field experiments in semi-arid and Mediterranean sites. This resistance to precipitation change is most likely explained by the magnitude of manipulative treatments lying well within the “climatic comfort zone” to which the component species were adapted. This can also be seen in our study in which significant differences in vegetation response variables were found between the −40% precipitation treatment and the increased precipitation or control groups, but not between the −20% treatment and the increased precipitation or control groups.

In this study, significant differences in the mean height and aboveground biomass among precipitation treatments were observed from the first year of the field experiment. The significant inter-annual variability of density indicates that the change in plant individual densities is also sensitive to natural precipitation variation. However, the response of species richness to precipitation manipulation seemed less sensitive, as significant differences in richness among precipitation treatments were not detected within each year but were detected across the three years. Baéz et al. (2013) found that species richness generally increased or was unaffected by water addition or drought in native grassland and grass–shrub ecotone vegetation during a 5-year manipulation experiment. However, Evans et al. (2011) found that the species number in a semi-arid shortgrass steppe was lower in drought treatments than the control during the earlier period of an 11-year drought manipulation and higher than the control in the later period. Thus, richness needs to be investigated over a longer experimental period, since the survival and colonization of drought tolerant plants and the growth of drought-intolerant species occur over a longer time (Suttle et al., 2007; Baéz et al., 2013).

A lot of the changes in soil properties are linked to the changes in biomass and cover of plant species in semi-arid or arid grasslands with secondary succession (Lozano et al., 2014). However, the asynchrony in plant and soil responses to precipitation manipulation was found in this study and other study (Wilcox et al., 2016). In our study, the difference in aboveground biomass between increased and decreased precipitation treatment in three years' experiment might be not enough to result in the difference in most of soil property. While in the study of Wilcox et al. (2016), shifts in plant species and functional composition may be an important consideration for understanding the stability of grassland soil C and N pools after increased precipitation of 25 years. Therefore, it is desirable to assess the species regeneration and community composition to precipitation variation through long-term experiments.

4.2. Effects of precipitation alteration on soil respiration and labile carbon and nitrogen pools

Soil respiration significantly increased along the experimental precipitation gradient (Fig. 3), similar to the changes in aboveground biomass and other plant growth properties (Fig. 2). Meanwhile, the relationship we observed between soil respiration and aboveground net primary productivity was comparable with other studies (Thomey et al., 2011; Vargas et al., 2012). Productivity, through the translocation of assimilated carbon to the rhizosphere, is a predominant factor determining soil respiration (Jongen et al., 2013). With significant changes in aboveground biomass (that is, the contribution of the vegetation to belowground CO_2 production), soil autotrophic respiration (including root respiration, mycorrhizal activity or root exudations) can be expected to be significantly different between precipitation treatments. In addition, a significant influence of precipitation treatments on SWC in the 0–20 cm depth was found in this study. Perennial grasses have shallow and lateral roots distributed in the surface soils and thus increased precipitation might have stimulated the root growth of

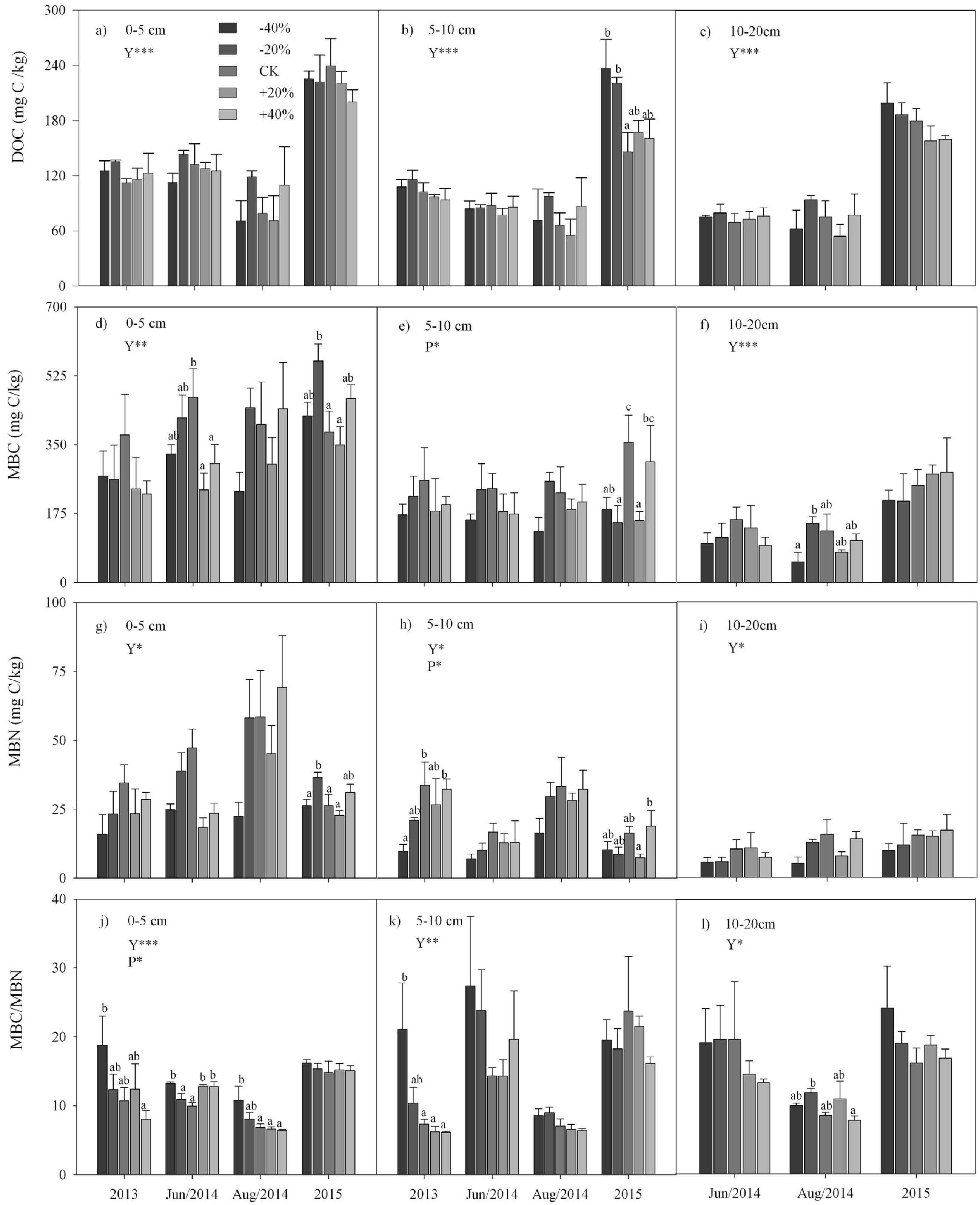

Fig. 4. Dissolved organic carbon (DOC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and ratio of MBC to MBN (mean ± S.E., n = 3, main panels) after precipitation alteration. Significant results of the two-way ANOVA on the effects of year (Y) and precipitation treatment (P) are shown. Significance: $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$. Bars with different letters within years represent significant differences based on post hoc testing at $p < 0.05$. −40%: 40% decrease in precipitation, −20%: 20% decrease in precipitation, CK: control, +40%: 40% increase in precipitation, +20%: 20% increase in precipitation.

grasses and caused a strong autotrophic respiration response. This is supported by other studies that reported that the promotion of soil respiration under increasing soil resources (water and nitrogen), to a great extent, is associated with soil autotrophic respiration (Su et al., 2016).

Moreover, there were also positive correlations between soil

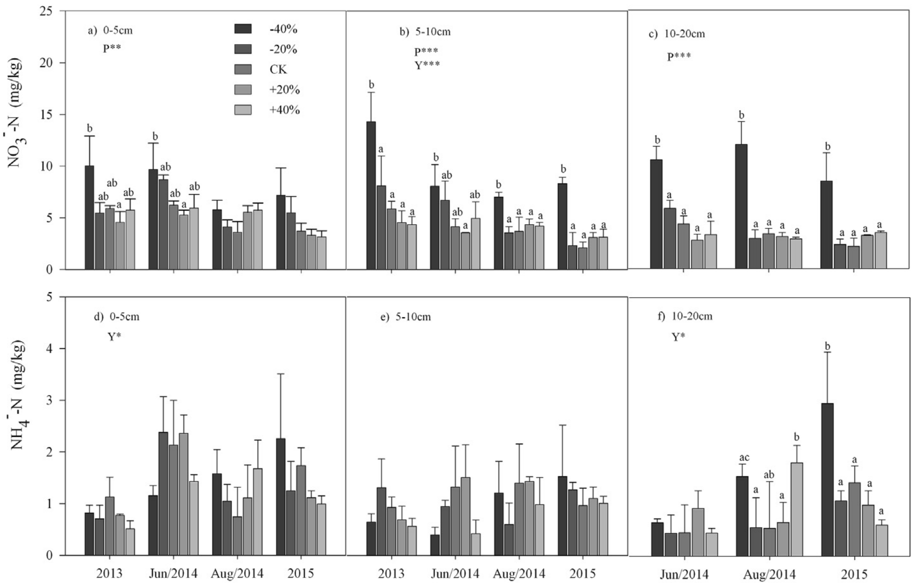

Fig. 5. NO_3^--N and NH_4^+-N (mean ± S.E., n = 3, main panels) after precipitation alteration. Significant results of the two-way ANOVA on the effects of year (Y) and precipitation treatment (P) are shown. Significance: $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$. Bars with different letters within years represent significant differences based on post hoc testing at $p < 0.05$. −40%: 40% decrease in precipitation, −20%: 20% decrease in precipitation, CK: control, +40%: 40% increase in precipitation, +20%: 20% increase in precipitation.

Table 2
Inter-annual variability of soil microbial relative abundances at the phylum level along the precipitation gradient.

Phylum	Year	Total	−40%	−20%	CK	+20%	+40%
		%					
Actinobacteria	2014	21.70	32.8	48.60	4.80	19.6	2.70
	2015	25.90	23.20	28.70	27.2	19.9	30.4
Bacteroidetes	2014	20.60	5.50	0.80	34.9	4.00	57.6
	2015	1.50	1.30	2.00	1.30	1.20	1.70
Proteobacteria	2014	18.50	20.00	15.30	21.7	16.5	19.1
	2015	34.80	42.80	29.80	28.7	47.1	25.6
Acidobacteria	2014	15.70	16.30	11.50	12.60	30.20	8.00
	2015	14.60	11.80	14.90	17.90	12.40	16.20
Verrucomicrobia	2014	7.20	7.10	1.00	12.4	12.8	2.70
	2015	3.10	2.70	3.10	3.20	2.40	4.10
Gemmatimonadetes	2014	5.90	9.90	11.90	0.40	5.50	1.60
	2015	6.30	6.80	7.10	5.20	5.70	6.70
Planctomycetes	2014	2.90	2.10	2.60	2.70	4.90	2.00
	2015	3.40	2.10	3.70	5.00	2.50	3.80
Chloroflexi	2014	1.30	1.10	2.90	0.50	1.50	0.30
	2015	3.40	3.10	3.70	3.40	2.70	4.10
Firmicutes	2014	0.90	1.00	0.30	0.90	0.80	1.50
	2015	2.20	1.40	1.90	3.90	1.80	2.30
Crenarchaeota	2014	1.00	0.20	1.10	2.60	0.60	0.30
	2015	1.50	1.90	1.60	0.80	1.50	1.60

respiration and MBN ($p < 0.01$; Table S2) in this study, suggesting that heterotrophic respiration may also respond to precipitation.

In this study, no significant influence of precipitation treatment on MBC and DOC were found, which was inconsistent with other studies where MBC, MBN and DOC increased with increased precipitation in temperate steppe and desert (Zhang et al., 2013; Zhou et al., 2013; Huang et al., 2015; Zhao et al., 2016). Labile organic C and N pools are closely associated with root productivity and aboveground plant communities (Rui et al., 2011). Thus, a large number of dead roots in the decreased precipitation treatments and active root turnover and root exudations in the increased precipitation treatments might contribute to the similar input of carbon found in this study. Meanwhile, stronger substrate leaching with increased precipitation is possibly related to the non-significant differences in DOC and MBC found between increased and decreased precipitation treatments in the study.

Additionally, microbial cells that remain intact at lower soil water conditions might have higher C/N due to osmoadaptation strategies (West et al., 1988). This might explain the higher MBC/MBN observed in the −40% precipitation treatment.

Precipitation treatment did not affect SOC content in this study, which was not consistent with another study that found that SOC concentration in the 0–10 cm soil layer increased with water addition in temperate desert (Huang et al., 2015). The SOC content depends on the balance between carbon input rate via organic residues and output rate via microbial decomposition. Although increased precipitation increased the aboveground biomass in our study, a large number of dead roots under decreased precipitation treatment might also contribute to a similar C input. Furthermore, Cotrufo et al. (2015) have shown that in finely textured soils, MBC is adsorbed quickly to clay and silt particles in large amounts, thus providing a substantial stabilization of soil C when levels of microbial activity are high in irrigated plots. In addition, reduced carbon mineralization rate and enhanced carbon use efficiency in drier soils could be largely attributable to reduced soil C loss (Zeglin et al., 2013; Zhao et al., 2016). Meanwhile, reduced precipitation also increases the formation of microaggregates in which the microorganisms are partly protected from the adverse effects of reduced soil moisture (Pujol Pereira et al., 2012). Therefore, the influence of precipitation treatments on soil C content was not significant. Wilcox et al. (2016) found no differences in total soil C between irrigation treatments after 25 years of chronic irrigation in tallgrass prairie.

4.3. Response of microbial community composition to precipitation alteration

In this study, the effects of precipitation treatments on bacterial abundances were evident in the wet year. For example, increased precipitation raised the relative abundance of *Bacteroidetes* and decreased the relative abundance of *Actinobacteria.* This result is consistent with that of Van Horn et al. (2014), who found that the addition of water resulted in a dramatic decline of *Actinobacteria* and an increase of *Bacteroidetes* in a polar Desert. Barnard et al. (2015) also found the relative abundance of *Actinobacteria* decreased with wet-up treatment in an annual grassland. Fierer et al. (2007) found that the abundance of *Bacteroidetes* was positively correlated with C mineralization rates. Thus, higher soil respiration rates in the increased precipitation treatments in the wet year were positively related to the higher relative abundance of *Bacteroidetes* in this study. Meanwhile, *Bacteroidetes* was not part of the dominant phyla in the dry year, and soil respiration was lower in the dry year than in the wet year.

The phylum-level community composition was similar to that of oligotrophic desert communities that contain a high abundance of

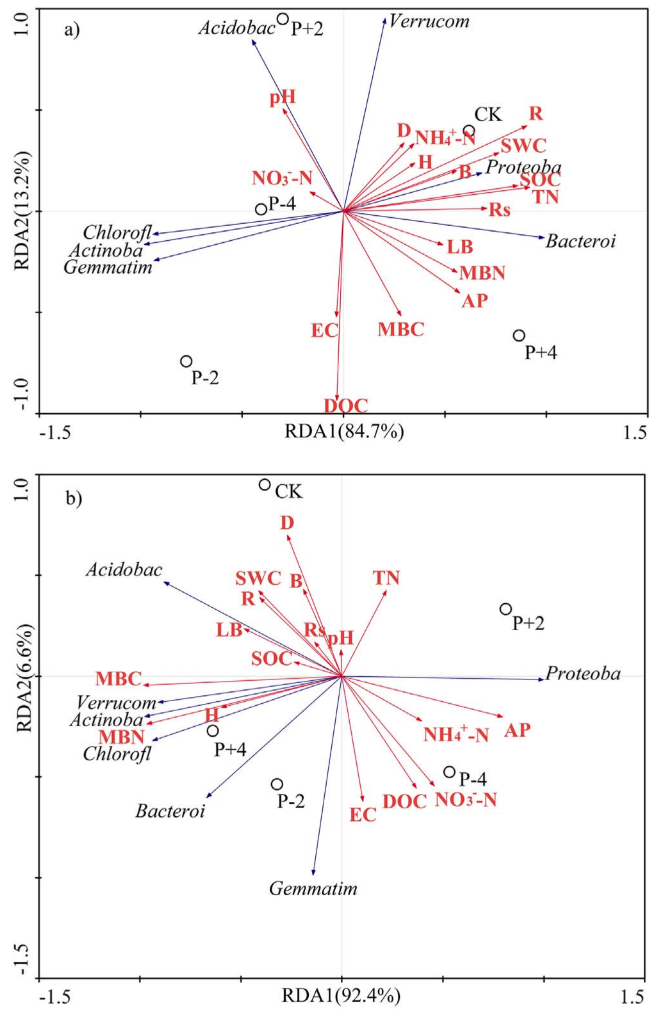

Fig. 6. Redundancy analysis (RDA) of the associations of soil bacterial community composition (as indicated by relative abundance of the main phylum) with environmental factors in 2014 (a) and 2015 (b). P-4, P-2, CK, P + 2 and P + 4 represent −40%, −20%, control, +20% and +40% precipitation treatment, respectively. R, richness; D, density; H, mean height; B, aboveground biomass; LB, litter biomass; DOC, dissolved organic C; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; Rs, Soil respiration; SWC, soil water content at a depth of 0–20 cm; TN, Total nitrogen; AP, available Phosphorus; SOC, soil organic carbon; EC, electrical conductivity.

Actinobacteria and *Acidobacteria* (Fierer et al., 2009). The increase in *Actinobacteria* in response to decreased precipitation suggests that *Actinobacteria* is probably adapted to dry and oligotrophic environments. Yao et al. (2017) pointed out that *Actinobacteria* and *Chloroflexi* were adapted to desiccation and thermal stress in arid conditions. However, the ecological categories of *Acidobacteria* are not very clear because other studies have found that their relative abundance increases (Barnard et al., 2015) or remains unchanged (Van Horn et al., 2014) in response to the addition of water. In this study, *Acidobacteria* was one of the dominant bacterial phyla, and no influence of precipitation treatments or year on their relative abundance were detected.

5. Conclusions

Shifts in precipitation regimes, especially in arid and semi-arid environments where water determines primary production, may strongly affect ecosystem dynamics. There were large responses of plant community properties, acute responses of bacterial community composition, and a lack of pronounced responses of labile carbon fraction and soil nutrients to three years of precipitation manipulation and inter-annual precipitation in a desert grassland. Additionally, the significant increase in soil respiration observed along the experimental precipitation gradient indicates the positive responses of root growth reflected by plant community properties and microbial activity reflected by bacterial community composition. Furthermore, ecosystem function depends on the relationship between the aboveground and belowground components when precipitation is altered.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.apsoil.2018.02.005.

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